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DEPARTMENT OF THE ARMY
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MULTIPLICATION AND VIRULENCE OF SEPTICEMIC
AND BLOOD PATHOGENS IN THE ORGANISM OF IN-
FECTED ANIMALS AND IN THEIR CARCASSES.

REPORT VIII. STUDY OF A MODEL OF EXPERI-
MENTAL INOCULATIONS WITH Salmonella
typhimurium

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D. Velyanov (with the assistance
in part of student circle mem-
bers T. Khinkov and S. Petrov)

Salmonella typhimurium was selected as the subject of
the investigations the results of which we set forth in the
present report. Its selection was occasioned by the following
two facts: first, among the types of Salmonella microorgan-
isms it is the most prevalent in nature and has the most
hosts and, second, it is isolated in the highest percentage in
the cases of toxinfections in humans.

What Salmonella typhimurium represents in comparison
with other Salmonella types can be seen very well from the
table given by Merchant and Packet [9], on the basis of the
data of Edwards and Bruner, relating to the USA for the period
1934-1947. It encompasses 6,381 outbreaks of Salmonella in-
fections caused by 20 Salmonella types in man and in 16 spe-
cies of domestic, experimental and wild animals and birds.

From the data in the above-mentioned table it can be
seen that out of a total of 6,381 outbreaks caused by 20 Sal-
monella types, most of them -- 1892 (31%) -- are due to Sal-

monella typhimurium. Moreover, this type has caused salmonella infections not only in man but also in the above-mentioned species of animals. As can be seen from the table, the other salmonella types have a considerably smaller range not only in respect of the number of animals affected but also in respect of the number of outbreaks registered. From a survey of Salmonella typhimurium infections it can be seen that, apart from domestic animals and a number of animals living in freedom or in semi-freedom, these infections affect rodents and birds -- a fact which shows the importance of this salmonella and of the infections caused by it from the viewpoint of the doctrine of the natural focus of infectious diseases.

The experience of using this salmonella type to control mice and other rodents yielded numerous facts which unequivocally emphasize the significance of cannibalism during an artificially induced epizootic among these animals as a most important factor in infections and reinfections.

As was emphasized at the beginning, Salmonella typhimurium is isolated in very high percentage in cases of food toxinfections in humans. According to the data of Kaufmann (cited by Yurkov et al. [8]), in Denmark it is isolated in 70% of cases, in Germany in 65%, in England in 55% and according to Shur [7] in the Soviet Union it is isolated in more than 50% of cases. In our country according to the data of Yordanov et al. [1] 60.8% of cases of food toxinfections are due to Salmonella typhimurium.

All these facts gave us reason to assume with great probability that not only the multiplication of Salmonella but also the intensification of their virulence take place not only in the carcasses but also in the meat of animals slaughtered perforce or when they are in an incubation period (in cases of salmonellosis caused by Salmonella typhimurium).

Set-up and Methods of the Investigation

The first part of the job of tracing the multiplication and changes in virulence of Salmonella typhimurium in the organism of infected animals in vivo and post mortem in the carcasses of dead animals was performed on white mice according to the method used in our preceding works [2, 3, 4, 5, 6, 10].

Changes in virulence were determined on cultures isolated from the liver of dead mice.

We calculated changes in the virulence of Salmonella typhimurium during the course of sickness on the basis of the

analysis of cultures isolated from the liver of white mice, killed in groups at different hours after inoculation, as indicated in the text and Table 3.

We used rabbits to trace the multiplication and virulence of Salmonella typhimurium in the musculature of animals under conditions similar to those of the forced slaughter of animals sick with salmonellosis or the slaughter of animals during an incubation period (prior to manifestation of the clinical picture of salmonellosis). The rabbits were each inoculated intravenously with 0.1-0.2 mm of 18- to 20-hour broth culture of Salmonella typhimurium, dissolved in 2-3 ml of physiologic solution. We killed a part of them early -- from 10 to 40 minutes after injection -- in order to simulate slaughter during an incubation period, while the rest of the rabbits we killed at the climax of their salmonellosis infection with the object of approximating the conditions of forced slaughter.

Immediately after slaughter we removed 0.500-0.800 kg of musculature which we chopped three times in a chopper and left at ordinary room temperature (18-20° C) in order to make the conditions of the experiment approximate the conditions and situation in which chopped meat is kept in practice and in which salmonella toxinfections most frequently break out. Using the chopped meat thus preserved, we traced the multiplication of salmonella and the concomitant microflora in it at different periods (at the moment of slaughter, 10, 24 and 48 hours thereafter) through gradual dilution in physiologic solution and culturing on ordinary and stained media, and at the same time we determined changes in the virulence of the isolated cultures by titration on white mice.

All the salmonella cultures isolated were identified by culturing on ordinary and stained nutrient media, the complete variegated series according to Kaufmann, and serologically.

Results and Discussion

Table 1 gives the results of tracing the rate at which and the extent to which Salmonella typhimurium multiplies in carcasses at a temperature of 18-22° C on the basis of microbiological findings in the carcasses of 34 white mice. From a review of what was found in blood smears it can be seen that at the moment of death no salmonella bacteria are discovered on the visual field or 2-3 bacteria are ascertained on each of a few visual fields in a few individual preparations. The same finding is ascertained at the 6th hour as well, becoming completely negative by the 12th and 18th hour. In prepara-

tions made at the 24th hour the finding is almost the same as at the moment of death and the 6th hour. In a few preparations 2-3-4 bacteria can be discovered across each of several fields. In subsequent hours the findings are negative. These data give reason to believe that prior to death the bacteriemic titer of Salmonella typhimurium is not high enough to be able to overcome the antibacterial properties of the blood or to assure more or less energetic multiplication of salmonella after death.

Table 1

RATE AND EXTENT OF POSTMORTAL MULTIPLICATION OF Salmonella typhimurium AT ROOM TEMPERATURE (18-22° C) ON THE BASIS OF FINDINGS IN CARCASSES OF 34 WHITE MICE

A Време след смъртта	В Кръв (брой на св.- монети на зрител- но поле)	С Черен дроб (брой на салмонелите на зрително поле)	Д Далак (брой на св.- монети на зри- телно поле)	Е Бъбрек (брой на салмонелите на зрително поле)
1 Момент на смъртта	от 0 до 2-3	от 0 до 17	0-7-15	0-10
2 6 ч.	0-3	92-171	42-12	56-82
3 12 ч.	0	176-294	117-182	122-191
4 18 ч.	0	219-424	194-392	192-374
5 24 ч.	0-3-4	321-340	253-295	206-217
6 36 ч.	0	302-456	290-324	196-280
7 48 ч.	0	288-426	394-426	201-452
8 72 ч.	0	0, а на някои по- леа до 50	0	0

Keys (by columns):

- A. Time after death
 - 1. Moment of death
 - 2. 6 hours
 - 3. 12 hours
 - 4. 18 hours
 - 5. 24 hours
 - 6. 36 hours
 - 7. 48 hours
 - 8. 72 hours
- B. Blood (number of Salmonella on visual field)
 - 1. from 0 to 2-3
- C. Liver (number of Salmonella on visual field)
 - 1. from 0 to 17
 - 8. 0, and on certain fields up to 50
- D. Spleen (number of Salmonella on visual field)
- E. Kidney (number of Salmonella on visual field)

This peculiarity of Salmonella typhimurium makes it like Listeria monocytogenes [16] and distinguishes it from the other septicemic pathogens, the objects of our preceding investigations [2, 3, 4, 5, 10].

Immediately after death from 0 to 17 bacteria per visual field are ascertained in smears from the liver. By the 6th hour this number increases 5- or 6-fold and in some cases even more, attaining an increase of 50- to 100-fold or more by the 24th-36th hour. By the 72nd hour a rapid drop is observed in the number of salmonella as the result of incipient lysis. We must note that after the 24th-36th-48th hour in almost every case there is observed an initially weak but later mass multiplication of extraneous septic flora.

In smears from the spleen made after death from 0 to 7-15 bacteria per field are discovered. After the 6th hour the number of bacteria fluctuates almost parallel with those of salmonella from the liver findings and reaches its maximum by the 48th hour. By the 72nd hour, as a result of the lytic processes that set in, no salmonella bacteria are discovered. Here, too, mass multiplication of extraneous microflora was ascertained by the 36th hour.

In the kidneys the number of salmonella shows the same tendency to rise from scattered bacteria at the moment of death to a maximum of 201 to 452 bacteria by the 48th hour.

From a comparison of the results obtained from the different organs it can be seen that multiplication of Salmonella typhimurium proceeds most energetically in the liver, more weakly in the spleen and kidneys while, as we pointed out supra, in the blood no multiplication of salmonella bacteria is noted [See Note]. All these data indicate that the fundamental avenue of infection can be only the peroral one and that the transmissive avenue for the transfer of infection can hardly be of any real value.

[Note]: The section on postmortal multiplication of Salmonella typhimurium was developed according to data from the work of student circle members in the Chair of Microbiology of the VVMH [Vishh veterinarno-meditsinski institut; Higher Institute of Veterinary Medicine], Tinko Khinkov and Simeon Petrov.

From a comparison of these results with the results of our preceding works [2, 3, 4, 5, 6, 10] it can be seen that Salmonella typhimurium in respect of the extent and rate of its multiplication in carcasses ranks after Past. multocida, Past. pseudotuberculosis and Bact. pyocyaneum but before Erys. rhusiopathiae and List. monocytogenes.

Table 2 gives the results of tracing the changes in virulence of Salm. typhimurium in the organism of white mice

from inoculation to the moment of their death. As can be seen from the Table, in the course of the disease virulence rises gradually. By the 3rd and 6th hour it is 3-1/2 times higher than initial virulence; it increases 5-fold by the 48th hour and 7-1/2 fold by the 120th hour, attaining a rise of 30-fold during the death struggle. The same degree of virulence was also noted in the cultures isolated from mice at the moment of their death.

Table 2

CHANGES IN VIRULENCE OF *Salmonella typhimurium*
IN THE ORGANISM OF WHITE MICE AT VARIOUS
PERIODS AFTER INOCULATION

A	Време при заразяването	B	Вирулентност (изходна : $3 \cdot 10^{-3}$)
1 2 3 4 5 6 7 8 9	3 ч.	$8 \cdot 10^{-3}$	(нараства $3\frac{1}{2}$ пъти в сравнение с изходната)
	6 ч.	$8 \cdot 10^{-3}$	
	24 ч.	$6 \cdot 10^{-3}$	(нараства 5 пъти в сравнение с изходната)
	48 ч.	$6 \cdot 10^{-3}$	
	72 ч.	$4 \cdot 10^{-3}$	(нараства $7\frac{1}{2}$ пъти в сравнение с изходната)
	96 ч.	$4 \cdot 10^{-3}$	
	120 ч.	$4 \cdot 10^{-3}$	
	166 ч. (в агония)	10^{-3}	(нараства 30 пъти в сравнение с изходната)
	Момент на смъртта	10^{-3}	

Keys (by columns):

A. Time after inoculation

- | | |
|-------------|-------------------------------|
| 1. 3 hours | 6. 96 hours |
| 2. 6 hours | 7. 120 hours |
| 3. 24 hours | 8. 166 hours (death struggle) |
| 4. 48 hours | 9. Moment of death |
| 5. 72 hours | |

B. Virulence (initial: $3 \cdot 10^{-2}$)

- 1-2. ... (3-1/2 fold increase as compared with initial)
- 3-4. ... (5-fold increase as compared with initial)
- 5-6. ... (7-1/2 fold increase as compared with initial)
- 8-9. ... (30-fold increase as compared with initial)

The above data, obtained during a slowly proceeding septicemia, are of special interest since they are similar to those gained in some of our earlier investigations into the causative agents of acute infections -- *Pasteurella*, *streptococci* and *staphylococci* [2, 10].

In Table 3 we indicate the results of our investigation of changes in the virulence of Salmonella typhimurium in the carcasses of white mice dead of salmonellosis, kept at different temperatures.

Table 3

CHANGES IN VIRULENCE OF Salmonella typhimurium
IN CARCASSES OF WHITE MICE KEPT AT DIFFERENT
TEMPERATURES (INITIAL VIRULENCE OF STRAINS
3 . 10⁻³)

	A Време след смъртта	B Температура, при която са съхранявани трупчетата		
		25°C	15°C	0-4°C
1	Момент на смъртта	3 . 10 ⁻³	5 . 10 ⁻³	3 . 10 ⁻³
2	5 ч.	5 . 10 ⁻³	10 ⁻⁴	—
3	9 ч.	5 . 10 ⁻³	5 . 10 ⁻⁴	10 ⁻⁴
4	24 ч.	5 . 10 ⁻³	5 . 10 ⁻⁵	10 ⁻⁴
5	36 ч.	—	10 ⁻⁵	—
6	48 ч.	10 ⁻³	10 ⁻²	3 . 10 ⁻³

Keys (by columns):

A. Time after death

- | | |
|--------------------|-------------|
| 1. Moment of death | 4. 24 hours |
| 2. 5 hours | 5. 36 hours |
| 3. 9 hours | 6. 48 hours |

B. Temperature at which carcasses are kept

As can be seen from the data, the virulence values of Salmonella typhimurium rise markedly at a temperature of 25° C, dropping thereafter to a level near initial virulence.

At the moment of death virulence is 10-fold higher than initial virulence. By the 5th hour it has increased sharply 6,000 times, remaining at the same level by the 9th and the 24th hour. By the 48th hour it has dropped markedly to a level somewhat higher than that at the moment of death.

At a temperature of 15° C the virulence curve of Salmonella typhimurium takes on a profile similar to that which we described for Bact. pyocyaneum [5]. After a 300-fold increase by the 5th hour, it dropped significantly (60 fold as compared with initial) by the 9th hour, increasing again

markedly during the later hours and reaching a 3,000-fold increase by the 36th hour. By the 48th hour it dropped to the level of initial virulence.

At a refrigerating temperature (0-4° C) a maximum increase in virulence of 300 fold is attained at the 9th hour and is maintained at the 24th hour as well. Thereafter a rapid drop is noted.

The above-indicated data show that the changes in the virulence of Salmonella typhimurium are significantly dependent on surrounding temperature not only with regard to the maximum reached but also with regard to the time during which it is reached.

At 25° C the maximum increase of 6,000 fold is reached as early as the 5th hour after death, at 15° it is 3,000 fold and is reached at the 36th hour after death, while at refrigerator temperature it is barely 300 fold. In this regard Salmonella typhimurium shows relationships similar to those established by us in the case of Brys. rhusiopathiae [4].

Comparison of the data derived from tracing the changes in virulence of Salmonella typhimurium in the organism of infected animals during the course of the disease and in their carcasses after death categorically indicates that the pathogen of which carcasses are the source represents a far greater danger than the one emanating from the organism of the sick animals.

The great postmortal multiplication of Salmonella typhimurium in the tissues of dead white mice and the sharp rise in its virulence after death at a temperature of 25° C (up to 6,000 fold in comparison with initial virulence) assures the pathogen the optimum opportunity for it to be disseminated and circulated among rodents, insectivora, carnivora and omnivora living at liberty in nature owing to the cannibalism widely prevalent among them.

The results of our researches serve to explain the very interesting data of Edwards and Bruner set forth at the beginning of the present work which relate not only to the infectious spectrum of Salmonella typhimurium but also to its great dissemination.

The significant influence exerted by different temperatures on changes in the virulence of Salmonella typhimurium in the carcasses of experimental animals distinguishes it from Past. pseudotuberculosis and List. monocytogenes [3, 6] and

can be regarded as proof of its later phylogenetic appearance as a naturally focal pathogen.

To trace the course of multiplication and intensification of virulence of Salmonella typhimurium in the musculature of experimental animals killed during the phase of injection bacteremia (incubation period) and at the climax of the disease (forced killing), we conducted experiments on rabbits, the results of which we set forth below.

In tracing the multiplication of Salmonella typhimurium in chopped rabbit meat that had stood at room temperature, a progressively mounting increase in the number of salmonella was ascertained up to the 48th hour when we halted the investigation owing to the onset of decay processes already intensely detectible organoleptically.

In the case of rabbits killed during an incubation period, from 1000-2000 to 10,000 salmonella per g of product were ascertained in the chopped meat prepared immediately after slaughter. By the 10th hour the number thereof increased several fold to 30,000-40,000, and by the 24th hour up to 90 million bacteria per g, attaining the huge figure of about 30-40 billion bacteria per g of chopped meat by the 48th hour.

In the chopped meat prepared from rabbits killed at the climax of the disease we counted from 1200 to 10,000 salmonella per g of product at the moment of death. By the 10th hour the number thereof increased two or three fold (up to 25,000-30,000). By the 24th hour it increased markedly to around 200 million salmonella per g of chopped meat, going as high as 10 billion by the 48th hour.

As concomitant microflora we isolated Bact. coli, Staph. albus, a few Sarcina, pigment-forming cocci and bacilli from the group Subtilis - Mesentericus.

Here, in contrast to the rapidly multiplying septic microflora in the carcasses of mice dead of salmonellosis, the saprophyte microflora that we found which had got into the chopped meat during processing and preservation showed no tendency towards intensified multiplication.

In Table 4 we indicate the data of our research on the changes in virulence of Salmonella typhimurium in the chopped meat obtained from rabbits killed during an incubation period and at the climax of the disease, kept at ordinary room temperature.

Table 4

CHANGES IN THE VIRULENCE OF Salmonella typhimurium
IN CHOPPED MEAT OF RABBITS KILLED DURING INCUBA-
TION PERIOD AND AT CLIMAX OF THE DISEASE,
KEPT AT ROOM TEMPERATURE (18-20°)

I Време след заколюването	II Зайци, заклани в инкубационен период			III Зайци, заклани в разгара на за- боляването		
	A заяк, заклан 10 мин. след i. v. заразяване	B заяк, заклан 30 мин. след i. v. заразяване	C заяк № 1, заклан 40 мин. след i. v. заразяване	A заяк № 2	B заяк № 4	C заяк № 5
1 Момент на смъртта	$5 \cdot 10^{-3}$	$5 \cdot 10^{-3}$	$5 \cdot 10^{-3}$	$3 \cdot 10^{-3}$	10^{-3}	$2 \cdot 10^{-3}$
2 10 ч.	$5 \cdot 10^{-4}$	10^{-5}	$5 \cdot 10^{-5}$	$5 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$8 \cdot 10^{-6}$
3 24 ч.	$3 \cdot 10^{-4}$	10^{-4}	$5 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$8 \cdot 10^{-6}$	$4 \cdot 10^{-5}$
4 48 ч.	$8 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$8 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	$6 \cdot 10^{-5}$	$2 \cdot 10^{-5}$

Initial virulence of the strain of Salmonella typhimurium is $3 \cdot 10^{-2}$. For the rabbit killed 30 minutes after inoculation a strain of Salmonella typhimurium with initial virulence of $2 \cdot 10^{-2}$ was used.

Keys (by columns):

- I. Time after killing
 - 1. Moment of death
 - 2. 10 hours
 - 3. 24 hours
 - 4. 48 hours
- II. Rabbits killed during an incubation period
 - A. Rabbit killed 10 minutes after i. v. inoculation
 - B. Rabbit killed 30 minutes after i. v. inoculation
 - C. Rabbit No. 1 killed 40 minutes after i. v. inoculation
- III. Rabbits killed at climax of disease
 - A. Rabbit No. 2
 - B. Rabbit No. 4
 - C. Rabbit No. 5

The data referring to changes in virulence of Salmonella typhimurium in the chopped meat of rabbits killed during an incubation period show that virulence at the moment of killing is several times (4-6 times) higher than initial virulence. By the 10th hour it rises sharply 600 to 6000 times. Thereafter, by the 24th hour it decreases to 100-200 times above initial virulence, becoming equal to it by the 48th hour.

In the chopped meat obtained from rabbits killed at the climax of the disease (salmonellosis), virulence at the moment of death had risen 30 fold, i. e. it had reached the level which is found in cultures isolated at the moment of death from the carcasses of mice dead of salmonellosis. Thereafter it rose from 3700 to 6000 fold in comparison with initial virulence, beginning gradually to drop by the 24th hour and reaching the initial level by the 48th hour.

Comparison of the data set forth above shows us that in both cases (animals killed during the phase of injection bacteriemia and at the climax of the disease) the maximum increase in virulence is reached at the 10th hour and exhibits similar values.

All things considered, the values of the changes in virulence coincide and the curves obtained from them are of identical character. This gives us reason to believe that the same potential hazard to the health of the consumer lurks in the meat obtained from animals that are in an incubation period or those killed perforce at the climax of the disease.

Also of interest is the fact that the maximum multiplication of Salmonella typhimurium is reached by the 4th hour while the maximum increase in virulence is attained as early as the 10th hour, dropping already by the 48th hour to a level close to the initial level.

Our data relative to the multiplication of Salmonella typhimurium in chopped meat agree with the data of former authors bearing on this question. However, they explained the outbreak and course of salmonella toxinfections in man solely on the basis of the rapid multiplication of salmonella in foodstuffs of animal origin.

Incomparably more important is the fact, established for the first time by us, that apart from the multiplication of Salmonella typhimurium in chopped meat obtained from rabbits there also takes place a vigorous intensification of the pathogen's virulence. This fact casts an entirely new light on the question of the pathogenesis of toxinfections.

It is this fact which makes it possible to explain the reasons for the much shortened incubation period in cases of toxinfection in contrast to that in the specialized nosological units caused by salmonella (typhoid fever, paratyphoid A and B, paratyphoid of swine etc.). Whereas in the case of the latter the incubation period runs into days, in toxinfections it usually lasts for only hours.

This difference, in our opinion, can be explained principally by the extreme rise in the virulence of Salmonella typhimurium in the flesh of animals killed during an incubation period or at the climax of the disease.

In the light of the results obtained by us, further research on Salmonella must be aimed at a study of any possible changes in the physiological and antigenic-and-structural peculiarities of the offspring that have heightened their virulence.

On the basis of the facts set forth above, the question of the epidemiology and epizootiology of salmonella and salmonella-induced food toxinfections must be reconsidered, with the other more widespread salmonella also undergoing study from this point of view.

Conclusions

On the basis of the experiments here conducted the following conclusions can be drawn:

1. Simultaneously with the occurrence of intensive postmortal multiplication of Salmonella typhimurium in the tissues of experimental animals dead of salmonellosis, there also takes place a very great increase in the virulence of this microbe. In contrast to the tissues, no multiplication of salmonella is observed in the blood.

2. Salmonella typhimurium reproduces most energetically post mortem in the liver, attaining an increase of 50-100 fold between the 24th-36th hour after death. In the spleen the number of salmonella fluctuates in almost parallel fashion with that ascertained in the liver, the maximum being reached by the 48th hour. Multiplication in the kidneys is less vigorous.

3. In the course of the disease the virulence of Salmonella typhimurium gradually rises, attaining a 30-fold increase during the death struggle stage in comparison with initial virulence.

4. The postmortal increase in the virulence of Salmonella typhimurium is directly dependent on surrounding temperature. At 25° C it reaches its maximum increase of 6000 fold between the 5th and 24th hour. At 15° the maximum is 3000 fold and is reached by the 36th hour, whereas at refrigerating temperature (0-4° C) virulence increases barely 300 fold in comparison with initial virulence.

5. The great differences registered between passage virulence (during the disease) and postmortal virulence of Salmonella typhimurium indicate the great danger which the carcasses of animals dead of salmonellosis represent from the viewpoint of epizootiology, epidemiology and the doctrine of the natural focus of infectious diseases.

6. Salmonella typhimurium multiplies very intensely also in the chopped meat obtained from rabbits killed during an incubation period and at the climax of the disease, going as high as tens of billions of salmonella per g of product by the 48th hour.

7. It is established that the virulence of Salmonella typhimurium in the chopped meat kept at room temperature rises rapidly and sharply and reaches its maximum increase of 2000 to 6000 fold in comparison with initial virulence by the 10th hour.

The lack of significant differences between the maximum value attained by the virulence of Salmonella typhimurium in the chopped meat obtained from rabbits killed immediately after intravenous injection (during an incubation period) and that for rabbits killed at the climax of the disease indicates that the flesh of such animals constitutes the same potential hazard for the occurrence of salmonellosis toxinfections in humans.

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SUMMARY

The authors trace the postmortal multiplication and change in the virulence of Salmonella typhimurium in the organism of experimentally inoculated white mice during the disease and in their carcasses after death, as well as in the flesh of experimentally infected rabbits killed during the stage of injection bacteremia or at the climax of a case of salmonellosis.

To clarify the first question, research was conducted establishing that at room temperature a significant postmortal increase in the numbers of salmonella takes place in the tissues of mice dead of salmonellosis, whereas none is observed in the blood. It was established that during the disease there is a gradual increase in virulence which in the period of the death struggle goes as high as a 30-fold increase in comparison with initial virulence. After death there takes place an extraordinarily great increase in the virulence of Salmonella typhimurium (up to 6000 fold), which to a considerable extent is dependent on changes of the surrounding temperature. The slight increase during the course of the disease (up to 30 fold in comparison with initial virulence) and the marked increase after death (up to 6000 fold at a temperature of 25° C) emphasize the important role of carcasses as a far more dangerous reservoir and source of infectious outbreak than the organisms of animals sick with salmonellosis.

To clarify the second question, the authors traced the multiplication of Salmonella typhimurium and the change in its virulence in the chopped meat of rabbits killed during the stage of injection bacteremia (incubation period) and at the climax of the disease (forced killing). It is established that multiplication of Salmonella typhimurium takes place vigorously in the chopped meat at room temperature and the number thereof goes as high as tens of billions of bacteria per gram of chopped meat by the 48th hour. It is noted that the virulence of Salmonella typhimurium in the chopped meat increases to its maximum by the 10th hour, reaching an increase of 2000 to 6000 fold in comparison with initial virulence.

The approximately identical maximum values which the virulence of Salmonella typhimurium reaches in the chopped meat of both groups of experimental rabbits attest to the identical potential danger which the musculature of these animals represents in the occurrence of salmonellosis toxinfections in humans.